Amendment

Please cancel claim 34.

Please add the following new claim.

39. (New) The cell of claim 1 wherein the essential gene does not encode a trans regulatory element for the lethal gene.

Remarks

Claims 1-33 and 35-39 are pending. Claim 34 has been canceled. Claim 39 has been added to more clearly recite what applicants consider to be their invention. Claim 39 requires that the essential gene not encode a trans regulatory element for the lethal gene. Support for new claim 39 appears at least on page 19, especially on lines 3-5, where trans regulatory element is defined as a molecule or complex that modulates the expression of a gene. A copy of all of the pending claims is attached to this Amendment and Response in an appendix.

Restriction Requirement

In the Office Action mailed December 23, 1997, claims 30-35 were indicated as withdrawn from consideration as being drawn to a non-elected invention. However, there is no basis in the prosecution history for holding claims 30-35 withdrawn, and applicants respectfully request examination of these validly pending and elected claims. In the Office Action mailed October 18, 1996, a restriction requirement was set forth where the claims were divided into two groups; Group I, claims 1-29, drawn to an isolated microbial cell, and

Group II, claims 30-35, drawn to a method of vaccination. The Office Action noted (page 3, lines 5-9) that if "claim 30 were amended to require both an essential gene and a lethal gene in accordance with claim 1, then the restriction between these two inventions would be withdrawn." In response (see Response mailed November 18, 1996, paragraph bridging pages 4 and 5), applicants amended claim 30 just as suggested to require both an essential gene and a lethal gene. In the next Office Action, mailed April 15, 1997, this amendment was duly noted and the restriction requirement between the two groups of claims was withdrawn (see page 2, lines 15-17). In the latest Office Action, mailed December 23, 1997, claims 30-35 are not rejected. Since no basis exists, either on the record or in fact, to withdraw claims 30-35 from examination, applicants respectfully request an indication of allowability of claims 30-35 or some other action consistent with examination.

The Claimed Cells and Method

The present invention is a microbial cell having an Environmentally Limited Viability System (ELVS) such that the cell is viable in a permissive environment and non-viable in a non-permissive environment. The ELVS achieves this environmentally specified viability using two components, an essential gene and a lethal gene. Essential genes and lethal genes are specifically limited in the claims, and defined in the specification (see pages 11-17, especially pages 11 and 16), to refer to mutually exclusive genes having mutually exclusive effects. As recited in the claims, an essential gene is a gene whose expression is essential to the viability of the cell. A lethal gene is a gene whose expression is lethal to the cell. Thus,

a lethal gene according to the claims cannot be an essential gene, and an essential gene cannot be a lethal gene. The expression of the lethal gene and the expression of the essential gene, as recited in the claims, are also mutually exclusive. This distinction between essential genes and lethal genes is important for assessing the relevance of the publications cited in the rejections (see discussion below).

Election of Species

Although the Office Action mailed December 23, 1997, indicates that claims drawn to non-elected species should be canceled in response to the Office Action, applicants believe this is improper. Pursuant to 37 C.F.R. § 1.146 and MPEP §§ 809.02, (b), (c), and (e), claims drawn to non-elected species are only provisionally withdrawn from examination pending determination of whether generic claims are found allowable. If the generic claims are found allowable, the species claims should also be allowed. Accordingly, claims drawn to non-elected species remain pending. Claims 30-35 are discussed above.

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 1-4, 8-14, 16, 20, 23, 24, 27-29, and 37 were rejected under 35 U.S.C. § 112, first paragraph, on the basis that the specification is enabling only for the use of some *Salmonella* strains as a vaccine. Applicants respectfully traverse this rejection.

Applicants initially note that the rejection continues to be based on a misinterpretation of the claimed cells. The claimed Environmentally Limited Viability System (i.e. the regulated essential and lethal genes recited in the claims) is **not** a bacterial attenuation

system. Rather, the environmentally regulated expression of the lethal and essential genes results in cell **viability** in the permissive environment and cell **non-viability** in the non-permissive environment. Attenuation of virulent bacteria is completely different and represents a feature that can be **combined** with the claimed Environmentally Limited Viability System. Where appropriate, the claimed Environmentally Limited Viability System can be embodied in a host cell that has been attenuated. Such attenuation is well known and is thoroughly described in the specification.

It is not clear what is the basis for the present rejection. For example, the passage on page 4, lines 7-10 of the Office Action appears to object to the administration of virulent bacteria (such as *Salmonella*) as a vaccine composition. First, the claims do not require administration of a virulent strain of bacteria as a vaccine composition. Second, it is not clear how such administration relates in any way to enablement of the present claims. In this regard, applicants note that even if attenuation were required in some applications, the state of the art and the extensive guidance in the specification for producing attenuated bacteria provides the requisite enablement for such attenuation. Applicants submit that the reasoning of the present rejection is unfounded, and that, as a consequence, no *prima facie* case of lack of enablement has been established.

Rejections Under 35 U.S.C. § 102

Claims 1-3, 10-13, 16, 20, 23, 24, 27, 28, and 37 were rejected under 35 U.S.C. § 102(b) as being anticipated by Gerdes *et al.*, *Proc. Natl. Acad. Sci. USA* 83:3116-3120

(1986). Claims 1-3, 8, 10-14, 16, 20, 27, 28, and 37 were rejected under 35 U.S.C. § 102(b) as being anticipated by Gerdes *et al.*, *EMBO Journal* 5(8):2023-2029 (1986). Applicants respectfully traverse these rejections.

Gerdes *et al.* (PNAS) disclose (page 3119, second column, and Fig. 3) *E. coli* containing a *hok* gene linked to λP_R which is regulated by the temperature-sensitive λCI_{857} repressor. The *hok* gene is expressed only when the temperature is raised to 42°C and the λCI_{857} repressor is inactivated. Expression of the *hok* gene produces a highly toxic gene product which causes rapid cell death. Thus, *hok* can be considered a lethal gene. Gerdes *et al.* (PNAS) also separately discloses a *sok* gene regulated by the temperature-sensitive λCI_{857} repressor. The *sok* gene is expressed only when the temperature is raised to 42°C and the λCI_{857} repressor is inactivated. Expression of the *sok* gene regulates expression of the *hok* gene. Thus, the *sok* gene can be considered a regulatory gene. Gerdes *et al.* (PNAS) fail to disclose any environmentally regulated *essential* gene.

Gerdes *et al.* (EMBO) disclose (page 2024, second column, and Fig. 1) *E. coli* containing a *hok* gene linked to λP_R which is regulated by the temperature-sensitive λCI_{857} repressor. The *hok* gene is expressed only when the temperature is raised to 42°C and the λCI_{857} repressor is inactivated. Expression of the *hok* gene produces a highly toxic gene product which causes rapid cell death. Thus, *hok* can be considered a lethal gene. Contrary to the implication of the Office Action, Gerdes *et al.* (EMBO) fail to disclose a temperature

regulated *sok* gene. Accordingly, Gerdes *et al.* (EMBO) fail to disclose any environmentally regulated essential gene.

The claimed cells require both an environmentally regulated lethal gene **and** an environmentally regulated essential gene. Since Gerdes *et al.* (PNAS) and Gerdes *et al.* (EMBO) fail to disclose or suggest such a regulated essential gene, Gerdes *et al.* (PNAS) and Gerdes *et al.* (EMBO) fail to disclose each and every feature of the claimed cells. Accordingly, Gerdes *et al.* (PNAS) and Gerdes *et al.* (EMBO) fail to anticipate the claimed cells and method.

To the extent that the present rejection is based on the assertion that the *sok* gene is an essential gene, applicants note the following. As disclosed by Gerdes *et al.* (PNAS), the *sok* gene is a regulatory gene of *hok* gene expression (Gerdes *et al.* (EMBO) does not disclose any regulated form of the *sok* gene). Enclosed is a copy of Franch and Gerdes, *Molecular Microbiology* 21:1049-1060 (1996), which describes the mechanism of this regulation. The *sok* gene produces an RNA that is an anti-sense RNA to RNA transcribed from the *hok* gene. When both the *sok* and *hok* genes are expressed, *sok* RNA prevents translation of *hok* RNA (and thus prevents production of the lethal *hok* protein). The *hok* RNA is much more stable that the *sok* RNA. Thus, when production of both *sok* and *hok* RNA ceases (such as when the *hok*-bearing plasmid is lost), the *sok* RNA is degraded first allowing translation of *hok* RNA to produce *hok* protein and cell death. This relationship

between *sok* RNA and *hok* gene expression makes the *sok* gene a **regulatory** gene of the *hok* gene.

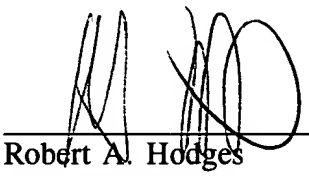
Applicants submit that a regulatory gene regulating expression of a lethal gene is clearly not the intended to be an essential gene within the claimed Environmentally Limited Viability System. The claimed ELVS is intended to operate with separate essential genes and lethal genes, each with a role to play. Regulatory genes are separately contemplated as a separate component. Although any regulatory gene (as defined in the specification) of a lethal gene might technically fit the definition of an essential gene (since the cell may die when the regulatory gene fails to repress expression of the lethal gene), such regulatory genes are clearly not intended to be considered essential genes within the context of the claimed cells. Applicants assert that the *sok* gene as disclosed by Gerdes *et al.* (PNAS) is a regulatory gene, not an essential gene, since it regulates expression of the *hok* gene and is not otherwise separately "essential" to the viability of the cell. Accordingly, Gerdes *et al.* (PNAS) fails to disclose cells as presently claimed.

New claim 39 emphasizes this distinction between regulatory and essential genes. Claim 39 provides that the essential gene does not encode a trans regulatory element of the lethal gene. A trans regulatory element is defined in the specification (see page 19, lines 3-5) as a molecule or complex that modulates the expression of a gene. Anti-sense RNA is specifically contemplated and described in the specification as an example of a trans regulatory element. Since the *sok* gene encodes an RNA that modulates expression of the

hok gene, the *sok* gene is specifically excluded as an essential gene in claim 39. Thus, claim 39 is not anticipated by Gerdes *et al.* (PNAS).

For all of the above reasons, applicants submit that the present claims are patentable. Allowance of claims 1-33 and 35-39 is respectfully solicited.

Respectfully submitted,



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Date: April 22, 1998

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I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Teresa R. Spratt

Date: April 22, 1998

Appendix

1. An isolated microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable in a permissive environment and non-viable in a non-permissive environment, the system comprising

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment; and

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment.

2. The cell of claim 1 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

3. The cell of claim 1 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal.

4. The cell of claim 1 wherein the essential gene, the lethal gene, or both, is carried on an extrachromosomal vector.

5. The cell of claim 4 wherein the lethal gene is carried on an extrachromosomal vector and expression of the lethal gene is regulated by an expression product of a regulatory gene.

6. The cell of claim 5 wherein the expression product of the regulatory gene inhibits expression of the lethal gene and is expressed or active only in the permissive environment.

7. The cell of claim 5 wherein the expression product of the regulatory gene induces expression of the lethal gene and is expressed or active only in the non-permissive environment.

8. The cell of claim 4 wherein the vector has two lethal genes.

9. The cell of claim 8 wherein the vector comprises pMEG-104.

10. The cell of claim 1 wherein the cell is a gram-negative bacterium.

11. The cell of claim 10 wherein the gram-negative bacterium is an enteric bacterium.

12. The cell of claim 11 wherein the genus of the enteric bacterium is selected from the group consisting of *Escherichia* and *Salmonella*.

13. The cell of claim 1 wherein expression of the essential gene is regulated by an expression product of a regulatory gene.

14. The cell of claim 13 wherein the expression product of the regulatory gene inhibits expression of the essential gene and is expressed or active only in the non-permissive environment.

15. The cell of claim 13 wherein the expression product of the regulatory gene induces expression of the essential gene and is expressed or active only in the permissive environment.

16. The cell of claim 4 wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed in the permissive environment and is not expressed in the non-permissive environment.

17. The cell of claim 16 wherein expression of the replication gene is regulated by an expression product of a regulatory gene.

18. The cell of claim 17 wherein the expression product of the regulatory gene inhibits expression of the replication gene and is expressed or active only in the non-permissive environment.

19. The cell of claim 17 wherein the expression product of the regulatory gene induces expression of the replication gene and is expressed or active only in the permissive environment.

20. The cell of claim 1 further comprising an expression gene wherein the expression gene encodes a desired expression product.

21. The cell of claim 20 wherein the desired expression product is an antigen.

22. The cell of claim 21 wherein the antigen is selected from the group consisting of bacterial antigens, viral antigens, plant antigens, fungal antigens, insect antigens, and non-insect animal antigens.

23. The cell of claim 1 for use as a vaccine, wherein the cell is viable when in the an animal and non-viable when outside of the animal, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal, and the lethal gene is expressed when the cell is outside of the animal and is not expressed when the cell is in the animal, wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

24. The cell of claim 23 further comprising an expression gene wherein the expression gene encodes a desired expression product.

25. The cell of claim 24 wherein the desired expression product is an antigen.

26. The cell of claim 25 wherein the antigen is selected from the group consisting of bacterial antigens, viral antigens, plant antigens, fungal antigens, insect antigens, and non-insect animal antigens.

27. A method of making a cell strain with environmentally limited viability comprising stably introducing into a cell

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the permissive environment and is not expressed when the cell is in the non-permissive environment;

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is in the non-permissive environment but not when the cell is in the permissive environment,

wherein the cell strain is viable in a permissive environment and non-viable in a non-permissive environment.

28. The method of claim 27 wherein the permissive environment comprises a temperature of about 37°C and the non-permissive environment comprises a temperature of less than about 30°C.

29. The method of claim 27 wherein the permissive environment is inside a warm-blooded animal and the non-permissive environment is outside a warm-blooded animal.

30. A method of inducing immunoprotection in a warm-blooded animal comprising administering to the animal a vaccine comprising a microbial cell comprising an Environmentally Limited Viability System, wherein the cell is viable when in the animal and non-viable when outside of the animal, the system comprising

(a) an essential gene, wherein expression of the gene in the cell is essential to the viability of the cell, the essential gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal; and

(b) a lethal gene, wherein expression of the gene is lethal to the cell and the lethal gene is expressed when the cell is outside of the animal but not when the cell is in the animal.

31. The method of claim 30 wherein the system further comprising an expression gene wherein the expression gene encodes an antigen.

32. The method of claim 31 wherein the antigen is selected from the group consisting of bacterial antigens, viral antigens, plant antigens, fungal antigens, insect antigens, and non-insect animal antigens.

33. The method of claim 30 wherein the cell is administered to mucosal surfaces of the animal.

35. The method of claim 30 wherein the essential gene, the lethal gene, or both, is carried on an extrachromosomal vector, and wherein the system further comprises a replication gene carried on a chromosome of the cell, the expression of which is required for replication of the vector, wherein the replication gene is expressed when the cell is in the animal and is not expressed when the cell is outside of the animal.

36. The cell of claim 5 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the lethal gene and wherein the expression product is not expressed or is inactive only in the non-permissive environment.

37. The cell of claim 13 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the essential gene and wherein the expression product is not expressed or is inactive only in the permissive environment.

38. The cell of claim 17 wherein the absence of a functional expression product of the regulatory gene derepresses expression of the replication gene and wherein the expression product is not expressed or is inactive only in the permissive environment.

39. The cell of claim 1 wherein the essential gene does not encode a trans regulatory element for the lethal gene.